EXHIBIT "A"

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of DENSLOW et al. Confirmation No: 3958

Application No. 10/663,561 Examiner: SALMON, Katherine. D.

Filed: September 15, 2003 Group Art Unit: 1634

For: DETECTING HORMONALLY ACTIVE COMPOUNDS

CERTIFICATE UNDER 37 CFR 1.8(a)
I hereby certify that this correspondence is being deposited either by facsimile to 571-273-8300 or with the U.S. Postal Service as First Class mail in an envelope addressed to Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on June 8, 2006

_____, Reg. No. 56,712
Nicholas A. Zachariades

RULE 132 DECLARATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Patrick Larkin, PhD, declare as follows:

1. I am one of the named inventors and am familiar with patent application No. 10/663,561 entitled " DETECTING HORMONALLY ACTIVE COMPOUNDS " (hereafter the '561 application) and the subject matter described therein.

2. I hold a PhD in Neuroscience and Molecular Biology and I am working in environmental toxicology I am presently employed as Vice President of Research and Development and Chief Scientific Officer at EcoArray Inc, Alachua, Florida.

3. I have authored or coauthored more than 16 scientific papers.

4. I have reviewed the Non-Final Office Action dated February 10, 2006 and references cited therein. I have been asked by patent counsel Zachariades to provide an explanation based on the claimed invention showing that the subject matter of the claims is not restricted to sheepshead minnow and largemouth bass fish but can be applied to other fish species without undue experimentation, unpredictability or requirement of high level of skill.

Claim 1 is copied below:

Claim 1. A method for detecting the presence of an agent having estrogenic or androgenic activity in a sample, the method comprising the steps of:

(A) providing at least one fish cell which was exposed to the sample;

(B) analyzing the at least one fish cell for expression of at least one gene wholly or partially encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO's: 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529 for identifying estrogen activity and SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 558 and 555 for identifying androgenic activity; and

(C) comparing the expression of the at least one gene in the cell compared to the expression of the at least gene in a control cell not exposed to the sample or an agent having estrogenic or androgenic activity, wherein a difference in the expression of the at least one gene in the at least one fish cell compared to the expression of the at least one gene in the control cell indicates that the sample contains an agent having estrogenic or androgenic activity.

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5. The Examiner rejects claims 1-7 and 10-32 are rejected in the instant Application in the February 10, 2006 Office Action " because the claimed invention does not reasonably provide enablement for any fish species or detection of genes partially encoded. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims."

6. I will address the Examiner's comments regarding the scope of enablement of the instant invention.

7. In the application we describe methods of detecting expression of genes in response to agents which cause estrogenic or androgenic activity in a sample. The endocrine systems of fish are highly conserved and work by essentially the same pathways. In all fish, various hormones like estrogens and androgens bind to their respective receptors, and this dimeric complex can then bind to specific promoter regions of various genes to induce their transcription. Estrogen and androgen receptors are highly conserved in sequence in many different species. For example, among various fish the homology can extend up to 90%. Because of this high conservation, estrogen and androgen receptors regulate the same set of specific genes in different species of fish. So, it is reasonable to expect that the same set of genes would be regulated by estrogen (and estrogen mimics) or androgen (and androgen mimics) in sheepshead minnows and largemouth bass as well as in other fish species. Along these lines, recent experiments we have

performed with yet another fish, the fathead minnow, support this contention and shows that the same set of genes are regulated.

8. The Examiner has rejected claims 1-7, 9-24 and 30-32 under 35 U.S.C. § 102(b) as being anticipated by Larkin *et al.* (*Marine Environmental Research* 2002 Volume 54 p. 395).

In the paper, we discuss the feasibility of applying array technology as a monitoring tool for detecting the presence and distribution of estrogenic compounds in coastal habitats using sheepshead minnows as our model. cDNA clones that were isolated from differential display, including vitellogenin α and β, vitelline envelope protein (ZP2), and transferrin, among others, were spotted on the macroarray. The results of these experiments demonstrated a characteristic expression pattern of estrogen responsive genes in sheepshead minnows exposed to17 β-estradiol (E2).

9. The paper in question (Larkin et al., 2002) does not anticipate the invention for several reasons. First, no sequences (fragments or full length) were disclosed in the paper. Without this information the study could not be replicated because the sequences were not in the public record. In addition, probe sequences that were used to make the gene chips were also not disclosed in the paper. Probe sequences are critical in making a gene chip, and without the probe sequences, the experiments that were discussed in the paper could not be replicated by other scientists. For example, even if a scientist knew the name of a gene (i.e. vitellogenin) that is a biomarker for estradiol, if the scientist used the wrong probe sequence within the vitellogenin gene to make a gene chip, there is a high probability that no positive response would be observed on the chips when the chip is hybridized with tissue extracted from tissue/cells etc. obtained from

animals that were exposed to estrogens, or compounds that mimic estrogens. None of this information in regards to probes that work (elicit a positive response) and probes that do not work (elicit a null response) for a given gene was taught or disclosed in the above mentioned paper.

Furthermore, we did not reveal the statistical analysis that we used to determine biomarkers that were identified in sheepshead minnows exposed for estradiol. A standard approach to identify biomarkers from microarray data sets has not been established in the field, so the rigorous approach we used could not be obvious. Our analysis used a combination of approaches which include employing various filtering algorithms, data mining techniques, and statistical programs (i.e. hierarchical clustering, SAM analysis, discriminant analysis) to identify genes that are biomarkers for specific compound.

10. I further state that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with my knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Patrick Larkin

Dr. Patrick Larkin

6/7/6

Date

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Nicholas A. Zachariades

RULE 132 DECLARATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Nancy D. Denslow, PhD, declare as follows:

1. I am one of the named inventors and am familiar with patent application No. 10/663,561 entitled " DETECTING HORMONALLY ACTIVE COMPOUNDS " (hereafter the '561 application) and the subject matter described therein.

2. I hold a PhD degree in Biochemistry and Molecular Biology and currently am working in environmental toxicology. I am presently an Associate Professor of Physiological Sciences at the University of Florida, Gainesville, Florida.

3. I have authored or coauthored 110 scientific papers, and two issued patents (Patent #5,650,299 -- Stem Cell Proliferation Factor, Michael Lawman, Pat Lawman and Nancy Denslow. July 22, 1997; Patent #5,981,708 – Stem Cell Proliferation Factor, Michael Lawman, Pat Lawman and Nancy Denslow. November 9, 1999).

4. I have reviewed the Non-Final Office Action dated February 10, 2006 and references cited therein. I have been asked by patent counsel Zachariades to provide an explanation based on the claimed invention showing that the subject matter of the claims is not restricted to sheepshead minnow and largemouth bass fish but can be applied to other fish species without undue experimentation, unpredictability or requirement of high level of skill.

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6. I will address the Examiner's comments regarding the scope of enablement of the instant invention.

7. We describe methods of detecting expression of genes in response to agents which cause estrogenic or androgenic activity in a sample.

The endocrine systems of fish are highly conserved and work by essentially the same pathways. In all fish, estrogens and androgens bind to their respective receptors to cause them to dimerize and then bind to promoter regions on genes that they control. This binding activates transcription of this set of genes. Estrogen and androgen receptors are highly conserved in sequence (for example among fish species the homology can extend up to 90%) and they regulate the same set of specific genes in different species of fish. So, that it is reasonable to expect that the same set of genes would be regulated by estrogen (and estrogen mimics) or

androgen (and androgen mimics) in sheepshead minnows and largemouth bass as in other fish. Along these lines, recent experiments we have performed with yet another fish, the fathead minnow, supports this contention and shows that the same set of genes are regulated.

8. A series of experiments was conducted in fathead minnows. This species of **fresh water** fish is totally unrelated to sheepshead minnow (salt water species) and largemouth bass (fresh water species). Sheepshead minnow and fathead minnow are both small fish and fractional spawners, but inhabit very different environments. Largemouth bass is a seasonal spawner, large fish that is freshwater. Thus the three species are quite different from each other.

For this set of experiments, fathead minnows were treated with ethinylestradiol (EE2), a strong pharmaceutical synthetic estrogen. The treatment was for 48 hr with three doses of EE2 (2 ng/L, 10 ng/L and 50 ng/L) mixed in the water. We examined the high dose by microarray analysis and found a long list of genes that were regulated in either a positive or negative manner, in the three tissues that were examined, including liver, gonad and brain. . Among the most highly regulated, we found the same genes that are listed in our patent including, vitellogenin, vitellogenin 3, estrogen receptors, StAR, signal peptidases, choriogenins, cathepsins, lipoproteins including apolipoprotein A1, liver fatty acid binding protein, fibrinogen and other proteins in the blood coagulation pathway, among others. The identified list from largemouth bass and sheepshead minnows were very similar.

We are in the process of analyzing for androgens using fathead minnows. Again we used 48 hr exposure to a potent androgen, trenbolone. We have not yet analyzed all of the data for gene expression changes, but spot checks suggest that the same list of genes as we found for largemouth bass and sheepshead minnows will be targeted. Thus we are certain that we can use the gene changes in largemouth bass and sheepshead minnow to predict changes in fathead minnow. The sequences for these genes in fathead minnow are not identical to the sequences in largemouth bass or sheepshead minnow. Nevertheless, there is sufficient similarity among the sequences to be able to identify the correct biomarkers.

9. The Examiner has rejected claims 1-7, 9-24 and 30-32 under 35 U.S.C. § 102(b) as being anticipated by Larkin *et al.* (*Marine Environmental Research* 2002 Volume 54 p. 395).

10. First, I will discuss the cited reference, of which I am a co-author. In the paper, we discuss the feasibility of applying array technology as a monitoring tool for detecting the presence and distribution of estrogenic compounds in coastal habitats using sheepshead minnows as our model. cDNA clones that were isolated from differential display, including vitellogenin α and β, vitelline envelope protein (ZP2), and transferrin, among others, were spotted on the macroarray. The results of these experiments demonstrated a characteristic expression pattern of estrogen responsive genes in sheepshead minnows exposed to 17 β-estradiol (E2).

11. Second, I will discuss why this paper bears no relevance to the instant invention. The paper in question did not identify the sequences of the genes that were differentially expressed as

is set forth in our application. A limited set of genes (4 genes) that are regulated were named but their sequences were not divulged. Other genes that were up and down regulated were not named. Of the named genes, three were up regulated by estrogen and 1 was down regulated. This information, by itself, was not specific enough to teach someone in the field about the invention. At the time this paper was published, the public databases contained sequences for only small fragments from each of the sheepshead minnow vitellogenins but no other sequences used in this invention were publicly known. That vitellogenin is responsive to estrogen has been known for a long time, but that it could be used in a panel of biomarkers to determine estrogenic compounds in the environment, was not known.

12. I further state that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with my knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nancy D. Denslow

Dr. Nancy D. Denslow

6-05-06

Date